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# Physical maps of the two circular plastid DNA molecules of the brown alga *Pylaiella littoralis* (L.) Kjellm

## Location of the rRNA genes and of several protein-coding regions on both molecules

**Susan Loiseaux-de Goër[1], Yves Markowicz[1], Jacques Dalmon[2], and Hélène Audren[1]**

[1] Laboratoire de Biologie Moléculaire Végétale, CNRS UA 1178, Université Joseph Fourier, BP 53, F-38041 Grenoble Cédex, France
[2] Laboratoire d'Hématologie, DRF, Centre d'Etudes Nucléaires de Grenoble, BP 85X, F-38042 Grenoble Cédex, France

**Summary.** Two circular molecules of different sizes, both belonging to the chloroplast DNA of the brown alga *Pylaiella littoralis*, have been observed by electron microscopy (Dalmon et al. 1983). Clone banks representing 86% of the small chloroplast circular DNA molecule (58 kbp) and 69% of the large circular DNA molecule (133 kbp) have been established and used as tools in the construction of physical maps. Two rDNA operons have been mapped in a very small inverted repeat on the large circular molecule. One 16S rRNA pseudogene and one split 23S rRNA gene have been mapped on the small DNA molecule, far apart from each other. Using heterologous probes, genes for ten different proteins have also been located on these maps. Their arrangement on the large molecule is different from that found in higher plants and algae. Probes from *rbcL*, *psbA* and *rps19* genes hybridize to several separated fragments. Two of them (*psbA* and *rps19*) hybridize to both types of molecules.

**Key words:** Brown algae – Plastid DNA – Genome heterogeneity – Plastid rRNA and protein genes

### Introduction

Very few plastid genomes from algae containing chlorophyll c have been analyzed. In fact, the plastid genomes of only two species have been studied so far: *Olisthodiscus luteus*, which is a unicellular alga of uncertain position, probably a *chloromonadophycea* (Billard, personal communication), and *Dictyota dichotoma*, a brown alga (Kuhsel and Kowalik 1985, 1987). In both these algae, the plastids are surrounded by the endoplasmic reticulum, but there is no direct connection between these organelles and the nucleus, as it is the case in true *Chrysophyceae* and in more primitive brown algae like *Pylaiella littoralis* (Bouck 1965). In *O. luteus*, the chloroplast genome is composed of a single type of many circular molecules, each having a size of 154 kbp and containing an inverted repeated region of approximately 22 kbp, on which the rRNA genes, the genes coding for both the large and small subunits of the ribulose-1,5-bisphosphate carboxylase oxygenase (*rbcL*, *rbcS*), and the gene coding for the 32 kd $Q_B$ protein (*psbA*) are located (Reith and Cattolico 1986). In *D. dichotoma*, 123 kbp circular molecules have been characterized. These molecules are composed of one large single-copy region, which includes the genes *psbA*, *psbE*, *psaA*, *psaB*, and *rbcL*, a small single-copy region of 42 kbp containing *psbB*, *psbC* and *psbD*, and a small inverted repeat region containing the rDNA operons. The gene organization of this molecule differs from that of higher plants (Kuhsel and Kowallik 1985, 1987). The existence of several different plastid circular DNA molecules has been reported for several chlorophyll c algae – *Pylaiella littoralis* and *Sphacelaria sp.* (Dalmon et al. 1983), *Monodus sp.* and *Ochromonas danica* (Reith and Cattolico 1986) – but these molecules have not been studied in detail.

In this paper, we present results concerning the heterogeneous plastid genome of the primitive brown alga *Pylaiella littoralis*. The plastids of this alga have morphological characters which are specific for primitive brown algae, the most evident being a prominent pear-shaped pyrenoid. We have constructed physical maps of the two major circular molecules which had been observed by electron microscopy (Dalmon et al. 1983), and located both the rRNA genes and ten other polypeptide-encoding regions.

*Offprint requests to:* S. Loiseaux-de Goër

## Materials and methods

*Shotgun cloning of plastid DNA*. Total plastid DNA was purified from axenic cultures of *Pylaiella littoralis*, as described by Dalmon et al. (1983). After complete digestion with EcoR1, BamH1, Sal1, or BamH1 and Sal1, according to the manufacturer's instructions, the fragments were integrated into the pBR325 (Bolivar 1978) dephosphorylated EcoR1 site or into pAT153 (Twigg and Sherratt 1980) cleaved with BamH1, Sal1, or BamH1 and Sal1. The transformation of HB101 cells and the selection of transformed clones were performed according to Maniatis et al. (1982). Cloned fragments were isolated, analyzed, and screened by hybridization with nick-translated whole plastid DNA from *P. littoralis* using classical methods described by Maniatis et al. (1982).

*DNA/DNA hybridization*. The transfer of DNA fragments after separation by electrophoresis through vertical agarose gels was done using Pall or Gene Screen Plus nylon membranes, which were often utilized for several successive hybridizations. All hybridizations were done at 65 °C (for homologous hybridizations) and 42 °C (for heterologous hybridizations), according to the manufacturer's instructions. These include overnight prehybridization and hybridization in a medium containing either 1% SDS and 1 M NaCl for Gene Screen Plus membranes, or 5 x Denhardt's buffer, 5 x SSPE (0.9 M NaCl, 50 mM $NaH_2PO_4$, 5 mM EDTA), and 0.2% SDS for Pall membranes. The membranes were then washed twice for 30 min each at 65 °C or 42 °C in 2 x SSC (0.3 M NaCl, 30 mM sodium citrate) and 1% SDS, and twice for 30 min each in 0.1 x SSC and 0.1% SDS at room temperature. The probes were prepared from plasmid DNA (described below) using conventional techniques. Inserts were isolated twice from agarose gels by the DEAE cellulose elution method (Dretzen et al. 1981) in order to minimize cross contaminations by other restriction fragments, and were labeled using Amersham's nick-translation kits and [$\alpha^{32}$P]-dCTP (Amersham, 3,000 Ci/mmole). Filters were exposed to Kodak X-Omat AR films for one night to several days.

*Heterologous probes*. The following DNA fragments were prepared using conventional techniques: an EcoR1-Sal1 subclone of pCrmCE1 containing the entire *Chlamydomonas reinhardtii* mitochondrial CO1 gene (gift of G. Michaelis); an intragenic 1.15 kbp PvuII-EcoR1 fragment from the spinach 16S rRNA gene, isolated from pSocB10 (Briat et al. 1982); three HindIII restriction fragments isolated from the pSocB6 spinach clone (Audren and Mache 1986) – a 1.5 kbp fragment containing 330 bp of the plasmid pBR322 (HindIII-BamH1) and the first 1,000 bp of the spinach 23S rRNA gene, an intragenic 1.1 kbp fragment from the 23S rRNA gene, and a 1.4 kbp fragment containing the last third of the 23S rRNA gene as well as the 4.5S and 5S rRNA genes; a *rbcL* intragenic 890 bp HindIII fragment and a *psaB* intragenic 1.1 kbp BamH1 fragment, isolated from the *C. reinhardtii* pCP43 clone (gift of J. D. Rochaix); a *psbA* intragenic 700 bp Hinf1 fragment, isolated from

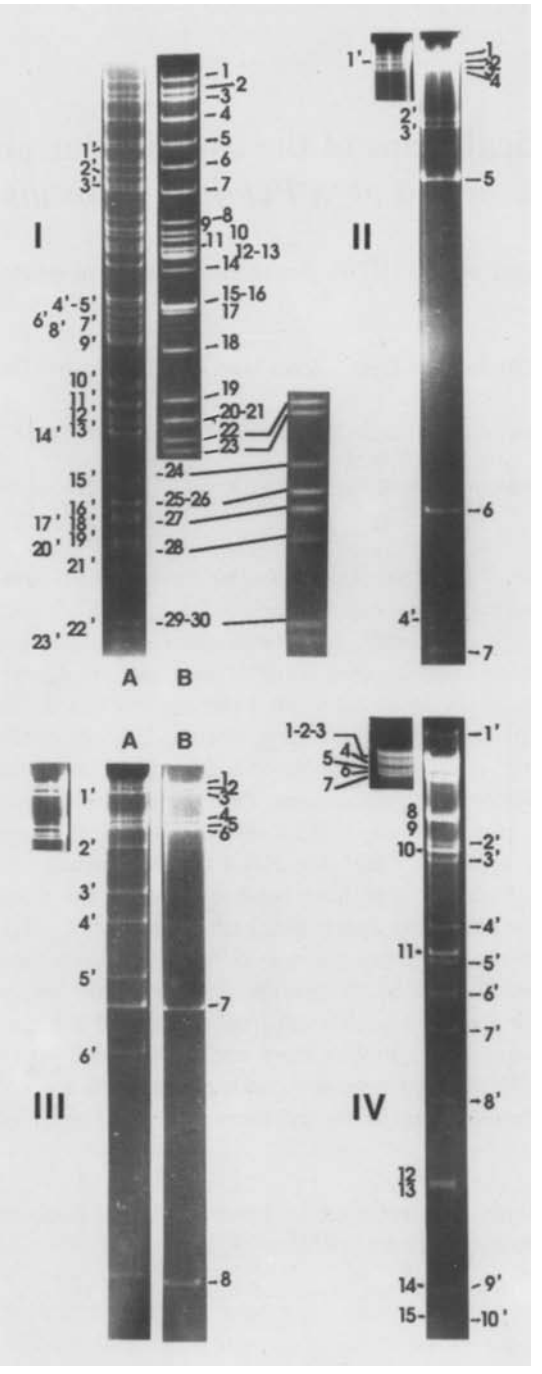


**Fig. 1 I, II, III, IV.** UV electropherograms of isolated plastid DNA from *P. littoralis* cleaved by different restriction enzymes. Fragments belonging to the large circular molecule are numbered *1, 2, 3* ...; fragments belonging to the small molecule are numbered *1′, 2′, 3′* .... **I** DNA cleaved by EcoR1. *A* DNA from the heavy part of the plastid peak; *B* DNA from the whole plastid peak. **II** DNA cleaved by Sal1. On the *left*, the upper part of the photograph was underdeveloped in order to distinguish between the different high molecular weight fragments which were overexposed. **III** DNA cleaved by BamH1. On the *left*, underdeveloped photograph of the upper fragments. *A* DNA from the heavy part of the plastid peak; *B* DNA from the light part of the plastid peak. **IV** DNA double-digested by BamH1 and Sal1. On the *left*, underdeveloped photograph of the upper fragments. Note that in **I** *B*, fragment E19 corresponds to the superposition of E19 and E10′, E20-21 to E20-21 and E11′, E23 to E23 and E13′, E24 to E24 and E15′, E27 to E27, E16′, E17′ and E18′, and E28 is enlarged by E19′ and E20′. This renders the evaluation of stoechiometries difficult

**Table 1A, B.** Numbering and molecular sizes (in kbp) of BamH1, BamH1-Sal1, Sal1 and EcoR1 restriction fragments of the two different *P. littoralis* plastid DNA molecules. BamH1 and Sal1 fragments which are not cut by the other enzyme in BamH1-Sal1 double digests are *underlined*. We consider 50 bp in estimating the size of small fragments and 200/500 bp for large fragments as an upper limit for error

**A** Small molecule

| BamH1 | | BamH1+Sal1 | | Sal1 | | EcoR1 | |
|---|---|---|---|---|---|---|---|
| B1′ | 24.9 | BS1′ | 24.15 | S1′ | 33.9 | E1′ | 7.8 |
| B2′ | 10.7 | BS2′ | 7.58 | S2′ | 12.1 | E2′ | 6.35 |
| B3′ | 7.58 | BS3′ | 6.73 | S3′ | 10.9 | E3′ | 6.05 |
| B4′ | 6.35 | BS4′ | 4.85 | S4′ | 0.95 | E4′ | 3.1 |
| B5′ | 4.85 | BS5′ | 4.15 | | | E5′ | 3.08 |
| B6′ | 3.5 | BS6′ | 3.5 | | | E6′ | 3 |
| | | BS7′ | 3 | | | E7′ | 2.9 |
| | | BS8′ | 2.18 | | | E8′ | 2.8 |
| | | BS9′ | 0.95 | | | E9′ | 2.7 |
| | | BS10′ | 0.75 | | | E10′ | 2.35 |
| | | | | | | E11′ | 2.1 |
| | | | | | | E12′ | 1.98 |
| | | | | | | E13′ | 1.9 |
| | | | | | | E14′ | 1.8 |
| | | | | | | E15′ | 1.5 |
| | | | | | | E16′ | 1.3 |
| | | | | | | E17′ | 1.25 |
| | | | | | | E18′ | 1.25 |
| | | | | | | E19′ | 1.1 |
| | | | | | | E20′ | 1 |
| | | | | | | E21′ | 0.95 |
| | | | | | | E22′ | 0.6 |
| | | | | | | E23′ | 0.6 |
| | | | | | | E24′ | 0.6 |
| 57.88 | | 57.84 | | 57.85 | | 58.06 | |

**B** Large molecule

| BamH1 | | BamH1+Sal1 | | Sal1 | | EcoR1 | |
|---|---|---|---|---|---|---|---|
| B1 | 32.3 | BS1 | 16 | S1 | 39.85 | E1 | 16.7 |
| B2 | 28.17 | BS2 | 15.5 | S2 | 31.5 | E2 | 13.4 |
| B3 | 25.35 | BS3 | 14.95 | S3 | 27.5 | E3 | 12 |
| B4 | 14.95 | BS4 | 14.05 | S4 | 24.7 | E4 | 9.75 |
| B5 | 13.5 | BS5 | 13.5 | S5 | 7.35 | E5 | 8 |
| B6 | 13.1 | BS6 | 12.6 | S6 | 1.5 | E6 | 6.75 |
| B7 | 4.27 | BS7 | 12.2 | S7 | 0.75 | E7 | 5.9 |
| B8 | 1.55 | BS8 | 9.05 | | | E8 | 5 |
| | | BS9 | 8.95 | | | E9 | 4.75 |
| | | BS10 | 7.35 | | | E10 | 4.55 |
| | | BS11 | 4.27 | | | E11 | 4.35 |
| | | BS12 | 1.55 | | | E12 | 4.25 |
| | | BS13 | 1.5 | | | E13 | 4.2 |
| | | BS14 | 0.95 | | | E14 | 3.95 |
| | | BS15 | 0.75 | | | E15 | 3.35 |
| | | | | | | E16 | 3.3 |
| | | | | | | E17 | 3.2 |
| | | | | | | E18 | 2.75 |
| | | | | | | E19 | 2.3 |
| | | | | | | E20 | 2.05 |
| | | | | | | E21 | 2.05 |
| | | | | | | E22 | 1.95 |
| | | | | | | E23 | 1.85 |
| | | | | | | E24 | 1.55 |
| | | | | | | E25 | 1.37 |
| | | | | | | E26 | 1.37 |
| | | | | | | E27 | 1.28 |
| | | | | | | E28 | 1.1 |
| | | | | | | E29 | 0.65 |
| | | | | | | E30 | 0.65 |
| 133.19 | | 133.17 | | 133.15 | | 134.32 | |

a spinach-cloned fragment (gift of H. J. Bohnert); fragments containing spinach structural gene sequences of the *petB* and *petD* genes, given to us by R. G. Herrmann; the spinach *atpA* intragenic TaqI fragment, the *atpB* intragenic SacI fragment, the *atpE* intragenic XbaI fragment and the insert containing the *atpH* gene, isolated from pWHsp210, pWHsp403/E1, pWHsp-403/E3 and pWHsp306/E2 respectively (gifts of R. G. Herrmann); a fragment containing the spinach *rps19* gene, isolated from pFT1 (gift of F. Thomas, of our laboratory); and intragenic fragments of the *rbcS* genes from *Anabaena* and tobacco, isolated from cloned fragments (gifts from R. Haselkorn and J. Fleck respectively).

## Results

### *Analysis of restriction patterns*

Upon complete digestion of *P. littoralis* plastid DNA by restriction enzymes, two different sets of fragments could be determined. One set is highly visualized, with related stoechiometries, as measured by densitometry of the UV fluorographs (not shown); the other set is poorly represented, but the fragments also show coherent stoechiometries between themselves, as measured by densitometry. The ratio of these two types of fragments varies according to the part of the plastid DNA peak obtained in CsCl gradients which is analyzed. DNA of the weakly represented fragment set is found mainly in the heavy part of the peak, which always shows a slight shoulder. These differences are shown in Fig. 1 (I A, B). We have numbered the weakly represented fragments 1′, 2′, 3′ ..., and the strongly represented fragments 1, 2, 3 ..., in decreasing size, in all figures and text. The sum of fragment lengths of weak or intense fragments, respectively, is always the same whatever enzyme was utilized. Hybridizations between cloned fragments from one clone bank and digests of whole plastid DNA by any enzyme showed

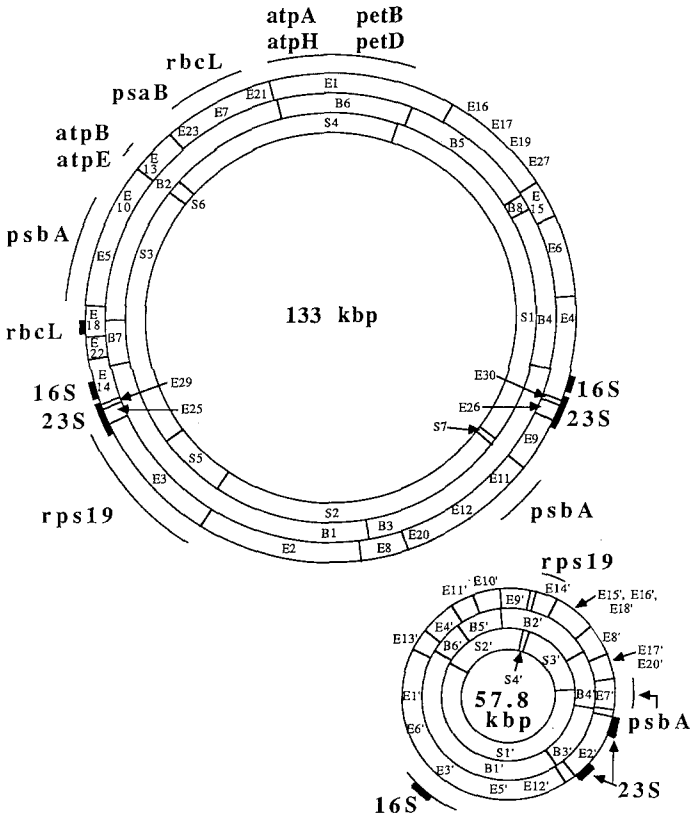


**Fig. 2.** Maps of the two plastid DNA molecules of *Pylaiella littoralis*. The maps show the restriction sites for BamH1, Sal1 and EcoR1, and regions hybridizing with different probes

that most of the weak or intense fragments cross-hybridize only to other weak or intense fragments, respectively. It is deduced that these fragments belong to two different categories of molecules that differ in their lengths and possess slightly different buoyant densities – 1.693 g/ml for the large molecule and approximately 1.698 g/ml for the small molecule. As judged by the relative densitometries of restriction fragments in different fluorographs, the plastid genome is composed of approximatively one-third 58 kbp molecules and two-thirds 133 kbp molecules. We have not been able to separate them from one another using CsCl gradients.

As shown previously (Dalmon et al. 1983), mitochondria are absent from our plastid preparations. In order to verify this, we hybridized a restriction fragment containing the entire *C. reinhardtii* mitochondrial gene coding for the subunit I of the cytochrome oxydase, which is a specific mitochondrial gene (Vahrenholz et al. 1985), to digests of the total *P. littoralis* plastid DNA. No hybridization occurred. We then hybridized this gene to a BamH1 digest of the DNA corresponding to the plastid DNA band from CsCl gradients, and to the region found between this band and the small nuclear DNA band ($\rho$ = 1.713 g/ml), which sometimes contaminates our routine preparations. After 3 days of autoradiography, a faint hybridization to a 2.3 kbp fragment was observed that was not visible under UV illumination of the ethidium bromide-stained gel, and not present in digests of DNA from purified plastids (not shown). This indicates the existence of traces of mitochondrial DNA that are usually eliminated when the plastid DNA is collected through a density gradient fractionator.

### *Mapping of the two molecules by BamH1 and Sal1*

Using the method of Fitch et al. (1983), the analysis of BamH1, Sal1 and BamH1 + Sal1 restriction patterns allowed us to construct a physical map for each molecule. The sum of the lengths of the weakly represented fragments is approximately 57.8 kbp in each digest (Table 1, A); that of the strongly represented fragments is approximately 133 kbp (Table 1, B). Ambiguities were resolved by hybridizing cloned EcoR1 fragments onto BamH1, Sal1, and BamH1 + Sal1 digests of plastid DNA. All hybridizations of BamH1 or double-digested BamH1-Sal1 fragments to EcoR1 restriction patterns, or of cloned EcoR1 fragments to BamH1 or Sal1 or BamH1-Sal1 restriction patterns confirm these maps. Circular maps are shown in Fig. 2.

### *Position of the rDNA operons*

Figures 3 (I B, II C, III B and IV) and 4 show the results of heterologous hybridizations between labeled frag-

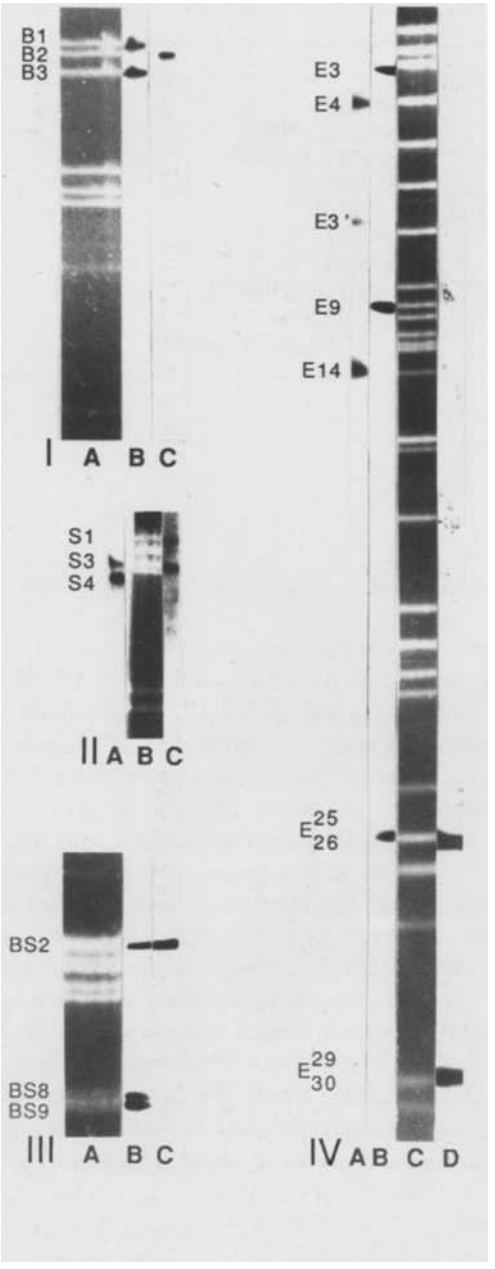


**Fig. 3 I, II, III, IV.** Hybridization patterns of *P. littoralis* plastid DNA, cleaved by different restriction enzymes, with heterologous probes, as described in Materials and methods. **I** Upper part of an electrophoregram of plastid DNA digested by BamH1 (*A*) hybridized with: the central part of the spinach 23S rDNA (*B*); the intragenic *C. reinhardtii psaB* fragment (*C*). **II** Upper part of an electrophoregram of plastid DNA digested by Sal1 (*B*) hybridized with: the intragenic fragment from the *C. reinhardtii rbcL* gene (*A*); the intragenic fragment from the spinach 16S rDNA (*C*). **III** Upper part of an electropherogram of plastid DNA double-digested by BamH1 and Sal1 (*A*) hybridized with: the whole spinach 23S rDNA gene and the cloned *P. littoralis* E12 fragment (*B*); E12 alone (*C*). **IV** Electropherogram of plastid DNA digested by EcoR1 (*C*) hybridized with: an intragenic spinach 16S rDNA fragment (*A*); the 3′ end of the spinach 23S rDNA (**B**); the central part of the spinach 23S rDNA (**D**). Hybridizations of these probes to the EcoR1 weak fragments are only visible for the 16S rDNA. Whole 23S rDNA would hybridize to the E2′ fragment if well represented

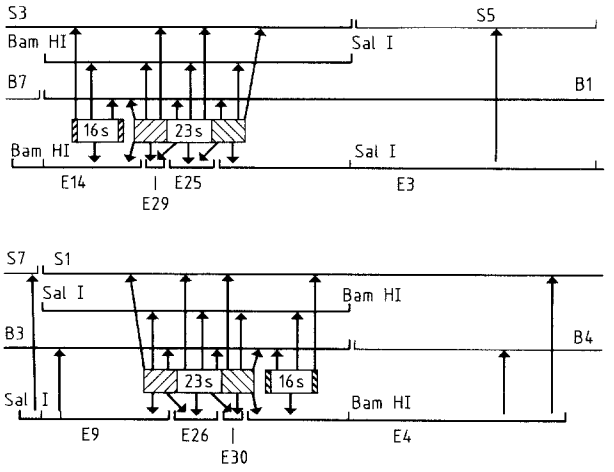


**Fig. 4.** Maps of the two rDNA operons located on the large circular plastid DNA molecule. *Arrows* indicate hybridizations between different parts of the spinach rRNA genes and the BamH1, Sal1, EcoR1 and BamH1-Sal1 fragments from *Pylaiella littoralis*

ments of spinach rRNA genes and *P. littoralis* plastid DNA. The 1.15 kbp internal fragment of the 16S rRNA gene (see Materials and methods) hybridizes to B1 and B3, S1 and S3, BS8 and BS9, and to the EcoR1 fragments E4, E14, which all belong to the large molecule, and, when weakly visualized fragments, which belong to the small molecule, are well represented, to S1′, BS1′, and E3′. The results obtained on the localization of the 16S rRNA genes onto the three EcoR1 fragments E4, E14, and E3′ will be published elsewhere. We will only mention here that the gene found on the E3′ fragment of the small molecule has a modified sequence and is a pseudogene (manuscript submitted).

As seen in Fig. 4, the region in the cloned E4 fragment not coding for the 16S rRNA is cleaved by BamH1, but not by Sal1, and hybridizes to B3, B4, and S1. Fragment E14 is also cleaved by BamH1, but not by Sal1, in double EcoR1-BamH1 and EcoR1-Sal1 digestions. Thus, both 16S rRNA genes are on the "BamH1 side" of fragments BS8 and BS9.

The beginning, central, and final parts of the spinach 23S rRNA gene (see Materials and methods) hybridize to B1 and B3, S1 and S3, and to BS8 and BS9, which belong to the large molecule, and to B3′, S1′, and BS2′, which belong to the small molecule (Figs. 3 and 4).

The first 1,000 bp of the 23S spinach gene hybridize weakly to E4 and E14, which both contain the 16S rRNA gene, and, with more intensity, to the double

**Table 2.** Summary of gene mapping hybridizations

| Gene[a] | Species | Filter-bound fragments hybridized[b] | Filter-bound cloned fragments hybridized |
|---|---|---|---|
| *atpA* | Spinach | E1 | pPliBS7 |
| *atpB* | Spinach | E13 | pPliE13, pPliS6 |
| *atpE* | Spinach | E13 | pPliE13, pPliS6 |
| *atpH* | Spinach | E1 | pPliBS7 |
| *petB* | Spinach | E1, B6 | pPliBS7 |
| *petD* | Spinach | E1 | pPliBS7 |
| *psaB* | *C. reinhardtii* | E7 | pPliE7 |
| *psbA* | Spinach | E5, E11[c] | pPliE11[c], pPliB4′ |
| *rbcL* | *C. reinhardtii* | E18, E7, B7, B2[c], BS11, BS6[c] | pPliE18, pPliE7 |
| *rps19* | Spinach | E3, E15[c] | pPliE15[c], pPliB2′, pPliE14′ |

[a] For details concerning probes, see Materials and methods
[b] The fragments belonging to the 57.8 kbp molecule were generally poorly represented
[c] Weak hybridizations

E29-E30 band. The central 1,100 bp of the spinach 23S rRNA gene hybridize to the double E25-E26 and E29-E30 bands. The end of the 23S rRNA gene hybridizes to E3 and E9. All three probes hybridize to E2′ when well represented.

The region which does not code for the end of the 23S rRNA gene, on the cloned E9 fragment, is cleaved by SalI, but not by BamH1, and hybridizes to B3, S1, and S7. The E3 fragment is also cleaved by SalI in double EcoR1-SalI digests, but not by BamH1, and therefore both 23S rRNA genes are on the "SalI side" of BamH1-SalI fragments 8 and 9. The maps of these regions are shown on Fig. 4.

Both rDNA operons are placed in reverse orientation on the large molecule (Fig. 2). Cross-hybridizations have been done between non-rRNA coding subfragments situated approximately 1 kbp upstream of the 16S rDNA and 2 kbp downstream of the 23S rDNA from one of these operons to the other. The absence of any hybridization shows that the inverted repeat rDNA region is not much larger than the rDNA operon itself. More experiments are needed to specify this point. Mapping of the 23S rRNA gene onto the cloned B3′ fragment, which belongs to the small molecule, and cross-hybridizations between the three different parts of the spinach 23S rRNA gene and subfragments of B3′ show that this gene is split by at least one intervening sequence of more than 3 kbp in the central part (not shown).

### *Mapping of defined genes on the genome*

The genes coding for ten different proteins have been localized using hybridizations with heterologous fragments of identified chloroplast genes. Probes are characterized as indicated in Materials and methods. Results are given in Table 2.

Three genes hybridize to several separated fragments. The *psbA* gene hybridizes strongly to E5, and weakly to E11 and to the cloned E11 fragment. This gene also hybridizes weakly to the cloned B4′ fragment of the small circular molecule. The *C. reinhardtii rbcL* intragenic fragment hybridizes strongly to E18 and weakly to E7. Both fragments are cloned and, after isolation, hybridize with the probe. The *rps19* probe hybridizes strongly to E3, faintly to E15 and the cloned E15 fragment, and strongly to the cloned B2′ and E14′ fragments. Hybridizations between labeled fragments containing the *rbcS* gene from *Anabaena* and tobacco, gave no positive results either on digested plastid DNA or on digested nuclear DNA. A similar result was obtained for *Olisthodiscus luteus* (Reith and Cattolico 1986) and other methods were used to localize this gene in the inverted repeat regions of the plastid DNA of this alga.

## Discussion

Substantial size heterogeneity in plastid DNA molecules has been reported for several chlorophyll c algae (Dalmon et al. 1983; Reith and Cattolico 1986), but has never been studied in detail. A small size heterogeneity of a few hundred base pairs has also been reported in the *Euglena gracilis* plastid DNA molecules (Jenni et al. 1981). The plastid DNA of *Pylaiella littoralis* is composed of two different molecules. One-third of the DNA is composed of small circular molecules which measure 53 to 59 kbp when examined by electron microscopy (Dalmon et al. 1983), and approximately 57.8 kbp as deduced from restriction analysis. The other two-thirds

are composed of larger circular molecules – 109.5 to 128 kbp as measured by electron microscopy and approximately 133 kbp as deduced from restriction analysis.

The presence of several cloned BamHI fragments of different lengths which hybridize to B4, and several cloned EcoR1 fragments of different lengths which hybridize to E4 and E6 (both being subfragments of B4) suggest that there is another small size heterogeneity group within *Pylaiella's* large molecule class.

The large molecule contains two rDNA operons which have a very short spacer between the 16S and the 23S rRNA genes (Markowicz et al. 1988) as do those of *Cyanophora paradoxa* (Bohnert et al. 1985; Janssen et al. 1987) and *Euglena gracilis* (Orozco et al. 1980). This is in contrast to previous observations on green algae and land plants, and in accordance with the size of a plastid rRNA precursor molecule (approximately 5.2 kb) observed in vivo (Loiseaux et al. 1980). These two rDNA operons are in opposite directions on the molecule, as in many land plants and algae. The two 23S rRNA genes are separated by 45 kbp and the two 16S rRNA genes by 75 kbp. Up to now, this is the only case, among algae, in which the order of the ribosomal RNA genes relative to the small single copy region (which in the case of *P. littoralis*, as in *D. dichotoma*, is rather large) is the same as in higher plants. On the small molecule, a 16S rRNA pseudogene is separated from the split 23S rRNA gene by at least 4 kbp and up to 27 kbp, as the cloned E3′ fragment, which carries the 16S rRNA gene, has not been precisely located on the BamH1-Sal1-1′ fragment.

The *rbcL* gene hybridizes strongly to a region situated approximately 4 kbp away from one of the rDNA operons, as in the case of *Olisthodiscus luteus* (Reith and Cattolico 1986), but on the large single-copy region and not to inverted repeated fragments. It hybridizes also to a 6 kbp fragment, 20 to 26 kbp further away, which also contains the *psaB* gene. It is to be noted that the *rbcL* and *psaB* genes are separated by only 700 bp in *Chlamydomonas reinhardtii* (Kück et al. 1987). More work is needed to find out if only part of the gene is located on this fragment, as hybridizations are weaker than on the other fragment. The *atpB* and *atpE* genes are close to one another as found in most plants, with the exception of *C. reinhardtii* (Woessner et al. 1987). The *rps19* gene hybridizes to a region located on the small single-copy region, as do several ribosomal proteins genes in *C. reinhardtii* (Schmidt et al. 1985). In higher plants, these genes are on the large single copy region. The *rps19* and the *psbA* genes also hybridize to fragments belonging to the small molecule. It is not known whether these fragments contain functional genes. On the whole, the localization of these ten genes to the large circular molecule differs from that found in all other plants, even from that in the brown alga *D. dichotoma*.

Recently, the probable existence of a heterogeneous population of rice plastid DNA molecules has been discussed (Moon et al. 1987). One of the rice plastid DNA molecules carries several defective genes. These genes belong to a cluster which has also been transferred to rice mitochondrial DNA. It is not known in this case, as in the case of *P. littoralis*, why a plant would maintain two kinds of molecules, one with defective genes, unless each type of molecule carries different non-defective genes, and thus both would be required to provide all the necessary plastid genes. It is also not known if these modified molecules have a relationship with the transfer of DNA from one genome to another. Clearly, more work is needed to resolve these questions.

As Kuhsel and Kowallik state (1985) "the few results from chlorophyll c containing algae are certainly not suitable for drawing further conclusions ...". This algal group is the most diverse and evolutionary complicated assemblage of different classes (see Bold and Wynne 1985), and various speculations have been made as to the origin and affiliation of these algae, without any really convincing arguments except for the probable fact that they arose from a secondary endosymbiotic event, as did *Euglena gracilis*. Their plastids probably evolved from an eukaryotic and not a prokaryotic symbiont (Gibbs 1981; Billard 1985). This, of course, does not solve the problem of clarifying the origin of their plastids.

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